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Testosterone therapy and bone quality in men with diabetes and hypogonadism: Study design and protocol

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ABSTRACT

Context: Type 2 diabetes mellitus (T2D) is often accompanied by male hypogonadism and both conditions are associated with increased risk for fractures. Testosterone (T) has been shown to improve the bone health of hypogonadal men but has not been tested in patients who also have T2D in addition to low T. To date, there is no treatment that is specifically recommended for bone disease among patients with T2D. This study will evaluate the effect of T therapy on the bone health of male veterans with low T who also have T2D.

Methods: This is a randomized double-blind placebo-controlled trial of 166 male veterans 35–65 years old, with T2D and hypogonadism, randomized to either T gel 1.62% or placebo for 12 months. We will evaluate the effect of T therapy on the following primary outcomes:1) changes in bone strength as measured by microfinite elements analysis (μFEA) using high-resolution peripheral quantitative computer tomography, 2) changes in bone turnover markers, and 3) changes in circulating osteoblast progenitors (COP) and osteoclast precursors cells.

Discussion: We anticipate that T therapy will result in improvement in bone strength owing to improvement in bone remodeling through an increase in osteoblastic differentiation and proliferation in patients with hypogonadism and T2D.

1. Introduction

An existing mutual influence between androgenic hormones and glucose metabolism has been proposed by studies showing that hypogonadism often accompanies type 2 diabetes mellitus (T2D) and vice-versa [1]. Men with T2D are commonly found to have low testosterone (T), while men with T deficiency are at increased risk to develop impaired glucose tolerance [1,2]. T therapy has been shown to improve glycometabolic control in hypogonadal men [1,3]. Furthermore, while the risk of developing T2D is 42% lower in men with high T levels [2], the odds of developing T2D is estimated to be 1.58 for every standard deviation decrease in T levels [4]. Thus, it is not surprising that as much as 64% of men with T2D were found to have low T [5,6].

Low T is associated with age-related decline in bone mass and increase in fractures in men, making androgen deficiency an important risk factor for osteoporosis. In fact, over half of elderly men with a history of hip fracture, were demonstrated to have T deficiency [7]. Through its conversion to estradiol (E2), T treatment is associated with a reduction in markers of bone turnover and a significant in increase bone mineral density (BMD) in young and elderly men with hypogonadism [8–10].

Type 2 diabetes mellitus (T2D) is associated with low bone turnover [11–14] and normal or high BMD, but paradoxically also with an increase in the risk for fractures [11]. There is even a suggestion to adjust the BMD T-scores of patients with T2D by −0.6 for women and −0.4 for men, and to consider treating them even if they are slightly below the FRAX-based intervention thresholds [11]. Considering the common association between T2D and hypogonadism, whether T (the standard of treatment for hypogonadism) will improve or worsen the skeletal health in men who also have T2D is unclear. T replacement in men with T2D

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and subnormal free T concentrations it has been shown that to increase in osteocalcin, indicating an increase in osteoblastic activity in the bone, but also a concomitant increase in bone breakdown, as displayed by the transient increase in C-Terminal telopeptide (CTx) [15]. However, to our knowledge, the best approach for treating bone disease in patients with T2D remains undefined.

This project will evaluate the effect of T therapy on bone quality in men who have hypogonadism and T2D. The central hypothesis of this 1-year randomized placebo-controlled study is that T therapy will result in improvement in bone quality owing to improvement in bone remodeling through an increase in osteoblastic differentiation and proliferation [16] in patients with hypogonadism and T2D. Thus, the objectives of this study are: 1) to determine the effect of T therapy on bone strength as assessed by finite element analysis (μFEA) men with T2D and hypogonadism compared to placebo using high-resolution peripheral quantitative computer tomography (HR-pQCT), 2) to determine the effect of T therapy on bone turnover markers in men with T2D and hypogonadism compared to placebo and 3) as exploratory aim, to examine the mechanism for the improvement in bone metabolism in response to T therapy in men with T2D and hypogonadism, by measuring circulating osteoblast progenitors and osteoclast precursors using flow cytometry technique.

2. Methods

2.1. Study design

This is a randomized double-blind placebo-controlled study protocol. Subjects will be randomized to either T or placebo at a ratio of 1:1. The study is currently conducted at the Michael E. DeBakey VA Medical Center (MEDVAMC) of Houston, TX in accordance with the guidelines in the Declaration of Helsinki for the ethical treatment of human subjects. The protocol was approved by the Baylor College of Medicine Institutional Review Board (ClinicalTrials.gov Identifier: NCT03887936) and the Veterans Affairs Office of Research and Development at the MEDVAMC.

2.2. Study population

2.2.1. Inclusion criteria

We will enroll 166 male veterans, 35–65 years of age, with an average fasting morning T level from 2 measurements of <300 ng/dl taken at least a day apart and having symptoms of hypogonadism as assessed using the quantitative androgen deficiency in aging male (qADAM) questionnaire [17]. Enrolled patients should have T2D, with a hemoglobin A1c (A1c) of <9.5%, fasting blood sugar of ≤180 mg/dl, body mass index (BMI) < 35 kg/m^2, and with T2D of ≤15 years duration to target men who have relatively less complications from long-term T2D.

2.2.2. Exclusion criteria

Subjects will be excluded from our study if they have 1) history of prostate or breast cancer; 2) history of testicular disease; 3) untreated severe sleep apnea; 4) ongoing illness that could prevent the subject from completing the study; 5) a hematocrit of >50% [17]; 6) prostate-related findings as: a palpable prostate nodule on digital rectal exam [18], serum prostate specific antigen [19] of ≥4.0 ng/ml or ≥3.0 ng/ml for African-Americans, International Prostate Symptom Score (IPSS) > 19 (severe); 7) on androgen therapy, or selective androgen receptor modulators; 8) on medications that affect bone metabolism such as: estrogen, selective estrogen receptor modulator as raloxifene, aromatase inhibitors, GnRH analogs, glucocorticoids with prednisone equivalent of least 5 mg daily for ≥1 month, anabolic steroids, phenobarbital and Dilantin; 9) use of bisphosphonates (i.e. risedronate, alendronate, zoledronic acid and pamidronate), within two years of study entry; 10) diseases that interfere with bone metabolism as hyperparathyroidism, untreated hyperthyroidism, osteomalacia, chronic liver disease, renal failure, hypercortisolism, malabsorption and immobilization; 11) current alcohol use of >3 drinks/day; 12) those with a history of deep vein thrombosis, pulmonary embolism, stroke or recent diagnosis of coronary artery disease. Because of the potential of being randomized to placebo, subjects with diagnosed osteoporosis, or with a T-score of less or equal to −2.5 assessed by dual-energy X-ray absorptiometry (DXA) at the lumbar spine, total femur or femoral neck, and those with a history of fragility fractures (spine, hip or wrist) will be excluded. Furthermore, patients with severe symptoms of hypogonadism defined as overall score of <10 in the qADAM questionnaire for androgen deficiency and those with total T level of <50 ng/dl will be excluded from study participation.

2.3. Procedures

2.3.1. Recruitment

Participants will be recruited mainly from the Endocrine, Primary Care and Urology Clinics at MEDVAMC, Houston TX. Recruitment strategies include active advertisement through posters and flyers, and direct mailings of printed materials to potential subjects. Potential subjects will be identified from direct referral by attending physicians or from patients referred for consult to the Endocrine Section for low total T levels <300 ng/ml and who meet the inclusion criteria. The records will be also reviewed for exclusion criteria. A member of the staff will then contact potential participants by a letter describing the study and introducing the investigators. Interested patients will be asked to return a postcard. We will access the Corporate Data Warehouse (CDW) data and vital status files through the Data Access Request Tracker (DART) to include real SSNs in order to identify potential eligible subjects for recruitment purposes. CDW data are accessible through VA Informatics and Computing Infrastructure (VINCI), an initiative to improve researcher's access to VA data and to facilitate data analyses while ensuring Veteran's privacy and security.

Individuals who express an interest in participation will undergo a brief telephone interview by a member of the research team. Interested patients who satisfy brief phone pre-screening eligibility assessments will be invited to visit our facility and discuss their potential participation in greater detail. During a 60 min long orientation session with members of the research team, detailed information will be provided regarding the aim of the study, and all the tests and measurements that they will undergo during participation. Verbal and written information about the potential benefits and risks of the study will be provided; their questions will be answered and any concerns they have will be addressed. If the individual is interested in participating, a screening evaluation will be scheduled. Informed consent to participate in the study will be obtained in writing by one of the investigators before any tests or measurements are performed. Prior to enrollment and randomization, the volunteers will undergo a detailed medical history, physical examination and a clinical laboratory testing.

2.3.2. Screening and baseline tests

Medical and social history and intake of medication will be obtained at baseline. Baseline T, luteinizing hormone (LH), follicular stimulating hormone (FSH), and prolactin will be obtained from fasting blood samples, which will be drawn between 8 and 10 a.m. to control for diurnal variation in hormone levels [17]. Two samples for T will be drawn at least a day apart and the average T will be calculated. Other blood tests include: comprehensive metabolic panel (CMP) with fasting glucose, A1c, fasting insulin, fasting lipid panel, complete blood count (CBC), PSA, TSH3, 25-hydroxyvitamin D (25OHD), parathyroid hormone (PTH), E2, sex-hormone binding globulin (SHBG).

Physical examination with a DRE will be performed. We will obtain height, weight, and BMI calculated as weight (kg) divided by the square of the height (m^2).

2.3.3. Primary outcomes

1. Microfinite element analysis (μFEA)-derived parameters such failure load and stiffness (which are indicators of bone strength) [20–26]; bone microarchitecture, geometry and volumetric BMD (vBMD) [27,28] on the radius and tibia and will be evaluated by HR-pQCT (XtremeCT II, Scanco Medical AG, USA, Inc) as previously described [29]. μFEA will be assessed using Scanco Medical's finite element analysis software with images generated using Image Processing Language provided to estimate the biomechanical properties of the bone as previously described [30]. The μFEA model will be subject to uniaxial compression and stiffness and failure load will be estimated. Using the latest generation of HR-pQCT scanner as described above (which allow for the highest resolution scans *in vivo*) we will evaluate cortical and trabecular microarchitecture of the distal tibia and radius [27]. Scans will consist of 110 CT slices of 9.02-mm region of interest. The volume of interest will be identified using the manufacturer's region-matching software, which relies on the periosteal contours. We will use the semi-automated software to segment cortical and trabecular regions based on the threshold-based algorithm. Microarchitecture parameters of cortical thickness (mm), trabecular thickness (mm), trabecular number (mm^{-1}), and trabecular separation (mm) will be determined, in addition to total, cortical and trabecular vBMD ($mgHA/cm^3$) [31]. For the cortical parameters, we will use the extended cortical analysis to derive cortical porosity (%), which is the volume of the resolved Harvesian canals and intracortical pore space normalized by the sum of their volume plus the cortical solid tissue volume [32].

2. Bone turnover markers. We will measure the bone turnover markers using enzyme-linked immunosorbent assay (ELISA). We will measure serum osteocalcin (OC), and procollagen I intact *N*-terminal (P1NP) as measures of bone formation; and C-telopeptide (CTX), tartrate-resistant acid phosphatase 5b (TRAP5b) as markers of bone resorption. Other markers include osteoprotegerin (OPG) and receptor activator of nuclear factor kappa B ligand (RANKL) and sclerostin.

3. Osteoblast and osteoclast precursors (exploratory aim): Using a modification of previously described methods [33–35], we will measure circulating osteoblast progenitors (COP) cells that are believed to reflect the bone formation at the bone marrow [33], and circulating osteoclast precursors using a revised method of Gossiel et al. [36]. Measurement of osteoblast progenitors and osteoclast precursors will be performed using Beckman Coulter Cytomics FC500 Flow Cytometer (Life Sciences, Indianapolis, IN).

2.3.4. Secondary outcomes

1. Areal BMD (aBMD) of the total body, lumbar spine and proximal left femur will be measured by DXA using Hologic Discovery (Hologic Inc., Waltham, MA, USA). BMD of the lumbar spine will be measured from L1 to L4. The nondominant hip will be used for studies on the proximal femur. The main region of interest on the proximal femur will be the total femur, and femoral neck.
2. Bone microindentation of the mid-anterior tibia will be performed using Osteoprobe Reference Point Indentation (RPI) technology (Active Life Scientific Inc., Santa Barbara, CA). This is the first and only available research instrument for direct measurement of tissue-level material properties of bone (cortical) and is used to obtain *in vivo* bone material strength index (BMSi) measurements, which is a direct measure of fracture resistance [37–40]. Main components include an impact mechanism, a displacement transducer, and a stainless steel probe with a 90° conical tip (375 μm diameter; <10 mm tip sharpness radius). The testing will be done at midshaft of the anterior tibia where soft tissue covering is minimal and is determined by calculating the midpoint from the medial border of the tibial plateau to the distal edge of the medial malleolus. After 1% lidocaine injection, the probe is inserted through the soft tissue and periosteum until residing on the bone surface. While keeping the device perpendicular, the measurement is actuated by slowly compressing the device's outer housing unit, compressing the internal primary spring until the trigger mechanism initiates an impact. The impact mechanism creates a force to drive the probe into the bone, while the displacement transducer measures indentation distance increase (IDI, μm) from impact. The IDI is converted by a computer to BMSi, defined as 100 times the ratio of the average IDI into a calibration phantom divided by the IDI measured in the bone. BMSi is calculated as the average of 12 measurements, separated by at least 2 mm. The indentations are small, around 375 μm, yet large enough to create microcracks in bone [37,39].

2.3.5. Other outcomes

1. Metabolic profile will be evaluated. We will assess lipid profile, A1C and homeostasis model assessment of insulin resistance (HOMA-IR) according to the formula (fasting insulin (microU/L) x fasting glucose (nmol/L)/22.5 [41].

2.3.6. Questionnaires

Questionnaires such as qADAM, IPSS, 7-day Physical Activity Recall (PAR), 3-day food diary and Food Frequency Questionnaire (FFQ) will be administered.

2.3.7. Follow up

Follow up visits will be done at 3, 6, and 12 moths as shown in Table 1. Interval medical history, physical examination, blood tests, aBMD by DXA, bone microarchitecture, volumetric BMD, failure load and stiffness by HR-pQCT, and microindentation measurements will be performed during follow up visits.

Symptoms will be assessed with IPSS and qADAM questionnaires and physical activity will be assessed by 7-day PAR. Food intake will be evaluated with 3-day food diary and FFQ. New and current medications and new medical problems will be recorded.

2.4. Intervention

There will be 2 arms of the study. Participants will be randomized to either T gel 1.62% or a matching placebo for 12 months.

2.4.1. Testosterone therapy

Based on previous studies, T gel was able to reduce markers of bone turnover, improve BMD [10] and bone microarchitecture [42,43] in men with hypogonadism. In this study, we will use T gel 1.62% at 2 pumps daily (40.5 mg) which is the starting dose suggested by the manufacturer and available at the MEDVAMC pharmacy. The matching placebo will be prepared by the pharmacy. Dosage adjustments will be

Table 1
Visits and schedule of tests.

Visits/tests	Baseline	3 Months	6 Months	12 Months
Medical history, physical exam including a digital rectal exam	x		x	x
T, CMP, fasting insulin, fasting lipid panel, A1c, CBC, PSA	x	x	x	x
LH, FSH, prolactin, TSH	x			
SHBG, E2, 25OHD, PTH	x		x	x
OCN, P1NP, CTX, TRAP5b, OPG, RANKL, sclerostin	x	x	x	x
Circulating osteoblast progenitors and osteoclast precursors	x	x	x	x
aBMD by DXA	x		x	x
vBMD, microarchitecture, μFEA by HR-pQCT	x		x	x
Microindentation	x			x
qADAM, IPSS, 7day-PAR	x	x	x	x
3 day food diary	x	x	x	x
FFQ	x			x

based on serum T levels, symptoms and the occurrence of side effects and will be performed by a physician co-investigator who is un-blinded to the treatment assignment but will not be involved in baseline or follow-up testing. We will aim to achieve total T levels between 500 and 700 ng/dl which is in the mid-range of normal (264–960 ng/dl) in the MEDVAMC laboratory. Dose adjustments will be done by increments or decrements of 1 pump to maintain the target T level. Repeat T measurements will be performed as outlined above from blood samples obtained between 2 and 4 h after application. A decrease in the dose by 1 pump will be done for patients who develop a hematocrit of >52%. T measurement will be performed 2 months after a change in dosage. For those who need adjustment because of elevation in hematocrit, a CBC will also be repeated 2 months after a change in dose. Otherwise, the schedule for follow-up testing will be as shown in Table 1. To maintain blinding, the physician making the dose adjustments will direct that a subject in the placebo group be treated similarly.

Subjects will be asked to take calcium carbonate 500 mg twice a day with 800 IU of vitamin D.

2.4.2. Randomization

T will be dispensed under double-blind conditions. Subjects will be randomized to T (n = 83) and placebo (n = 83) using random number generation; randomization list to be provided by the study biostatistician. The study coordinator will inform the MEDVAMC pharmacy and the biostatistician when a subject is ready for randomization. The T gel 1.62% and placebo gel will be provided in an identical form and dispensed by the MEDVAMC pharmacy. The pharmacy will complete a group assignment form, copies of which will be provided to the biostatistician and the unblinded investigator. Since the same biostatistician will be performing both randomization and analysis, in order to maintain the blinding during data analysis, he will assign letters A and B, which represent treatments, to each study subject ID and the research pharmacist will make a one-time decision whether A or B represents T or placebo. De-identified outcome data with the A and B designations will be provided back to the statistician for analysis. Lists of the participants and their treatment assignment will be kept by the pharmacy. The pharmacy will dispense the T and placebo to the study patient on a monthly basis and will maintain a record of the dosage dispensed. The study coordinator will contact participants every 2 weeks by phone during the first 2 months to discuss any concern in relation to the medication. The coordinator will document the dose of T that the subject is taking daily and any time the dosage is changed on a standardized form (though the coordinator will be blinded to the treatment status).

2.5. Safety monitoring and study termination

Our group has an established experience with clinical trials involving T therapy and related safety procedures [44]. All subject will be seen by a physician before beginning the study, and a physical exam with a DRE performed at baseline, 6 and 12 months. Lab tests (CBC, PSA, CMP and fasting lipid panel), symptom checklists will be completed and reviewed at months 0, 3, 6, and 12 (Table 1). New symptoms will be triaged to the PI. Individuals in the T treatment group who are unable to maintain T levels in the target range despite dosage adjustments will be dropped from the study. In addition, those who have persistently elevated hematocrit levels above 54% despite reduction in the dose of the study drug to as much as 50% of the weekly dose [17] will be asked to leave the study. Similarly, those who experienced an increase in PSA above 4 ng/ml -cut off that for African-American is set at 3 ng/ml-during any 6-month period [17], will be dropped from the study. Individuals dropped out from the study will be offered to follow-up at the Endocrine Clinic at the MEDVAMC or referred to their primary care provider for further management. Anyone with a bone loss of ≥6% at the spine or total femur over a 6-month period of observation or who develop fragility fracture will be dropped-out from the study and advised to follow-up with his regular physician or at the Endocrine Clinic. We will also monitor changes in liver enzymes (available in the CMP) and lipid levels. A Data Monitoring Committee appointed by the Veterans Affairs Clinical Science Research and Development board will convene every 6 months to review data on recruitment, adverse events and overall progress of the study.

2.6. Sample size/power analysis

Our sample size calculation utilized the changes in failure load (μFEA-derived) at the tibia in 15 men in our unpublished preliminary data (Table 2) with both low T and T2D randomized to either T or placebo. A sample size of 132, i.e. 66/group, will detect the minimum detectable difference in % change of 3.26% at the tibia between T and placebo with >80% power and two-sided alpha = 0.05. This sample size will also detect a 5.13% difference in failure load change at the radius between T and placebo. Likewise, basing on the result of our unpublished preliminary data (Table 2), the sample size will be adequate in detecting a difference in CTX changes estimated to be increased by 74.4% and the estimated 110% increase in osteoblast progenitors in the T treated group compared to no change (in both outcomes) in the placebo group. With an estimated drop-out rate of 20% or less, we will recruit 83/group, in order that the remaining 66 subjects/group will have adequate power to detect the estimated difference in % change in the outcomes.

2.7. Statistical analyses and data interpretation

Treatment associations with changes in the primary outcome (i.e. μFEA) over time will be modeled using linear mixed models with treatment as the grouping factor and measurement times as the repeated factor will be adjusted for covariates as age, and initial value. Additional covariates considered are: race, pack/years smoking, body mass index, duration of diabetes, medications, presence of diabetes complications, the Charlson index [45], changes in A1c, strength, lean mass, 25OHD hormonal levels, physical activity, macro and micronutrients intake, caloric intake. Changes in CTX and circulating osteoblasts precursors over time will be analyzed by linear mixed model adjusted for baseline values. Other secondary outcomes as BMSi, aBMD, HR-pQCT parameters (vBMD, bone area, cortical and trabecular thickness), biomarkers (sclerostin, RANKL, and OPG), hormonal levels and circulating osteoclasts will similarly be analyzed with adjustments as appropriate. We will perform follow up post hoc after the linear mixed model shows a significant omnibus test (i.e. $p < 0.05$). Log transformations will be consider when they will equalize variances. If we discover that the distributional assumptions required for the above analyses are not met by our data, even after standard data transformation, we will explore the use of more robust semi-parametric or non-parametric tests (e.g. rank tests). We will employ two powerful statistical methods to deal with the issues of non-compliance and missing values. These will be considered as alternative analyses unless the original analyses are not validated thereby; in which case these analyses will be promoted in importance in our results. First, if non-compliance in either arm results in missing values, we use multiple imputation (MI) to randomly generate 100 complete databases. Our statistical analyses will be repeated for these 100 databases, and the 100 estimates ± SE will re-combined (MIANALYZE) into one, less biased answer/result. MI and MIANALYZE procedures are available in SAS. Second, if non-compliance results in biased values (not missing values), non-compliance measures; for example, # of prescription refills for T, placebo and diabetic medications, as well other measures such as age, will be used as predictors in a non-parsimonious propensity scoring in order to adjust for these biases. Typically, the propensity scores (PS, probabilities) are computed as predicted values in a (non-parsimonious) logistic regression of two arms, as a binary outcome, on predictors. Weight for each record in the database are computed by a stabilized inverse probability method. Subsequently, our alternative analysis will be the weighted analyses using these weights.

Table 2
Power calculation based on a sample size of 166: 83 randomized to testosterone and 83 to placebo.

Aim	Primary outcome	Source	SD for % change Placebo n = 8	SD for % change Testosterone n = 7	Observed mean % change difference	Detectable %change difference for n = 66 per group and 80% power	Adequate power
1	FEA parameters: a) Failure load, Tibia	Preliminary data in men with low T + T2D	2.21%	4.30%	3.26%	1.7%	Yes
	b) Failure load, Radius		4.51%	9.30%	5.13%	3.6%	Yes
2	C-Telopeptide (CTX)	Preliminary data in men with low T + T2D	150.6%	150.6%	[a]74.4%	74%	Yes
3	Osteoblast progenitor	Cohen et al.	[b]55%	55%	110%	27%	Yes

Notes: The power analyses presented here take the form: given SDs, desired power (80%) and alpha, and planned sample size (66/group), what is the minimum detectable mean difference in % change between groups. Feasibility is judged by the observed difference being greater than this number.

[a] The changes in CTX in response to placebo in men with low T and T2D is unknown; we assume 0% change for the mean. Our preliminary data of response variability to T in hypogonadal men with T2D will be applied to the response in the placebo arm.

[b] Cohen et al., 2017 JBMR show a 221% ± 110% increase in osteoblast progenitors due to Teriparatide. We assume a response due to T of 50% of these values.

3. Discussion

T remains the standard therapy for hypogonadism and is expected to improve the bone health of hypogonadal patients [9,10,42,43], while to date, there is no therapy that is primarily targeted to treat bone disease of patients with T2D. However, considering the relationship between T2D and hypogonadism, whether the positive effects of T replacement are associated with improvement of bone quality in men who have T2D and hypogonadism is unclear. Using novel and state-of-the art techniques with complementary outcomes, this project is designed to fill this gap in information. The current project is the first randomized controlled trial (RCT) to assess the different aspects of bone quality in response to T therapy in hypogonadal men with T2D.

With the primary outcome of this study we will establish if T therapy in hypogonadal men with T2D will result in improvement in bone strength (as measured by μFEA). T2D is associated with normal or high BMD but paradoxically increased fracture risk [46]. As skeletal fragility in these patients is unrelated to bone quantity but a problem of bone quality, the use of HRpQCT will allow us to evaluate bone strength (by μFEA), the best indicator for bone quality. While some studies reported preserved microarchitecture in patients with T2D [47], others reported increased cortical porosity in these patients [32]. However, μFEA, microarchitecture and geometry of diabetic subjects who also have hypogonadism remains uncharacterized. These bone properties will be described at baseline and the changes that follow after T therapy will be evaluated in this study by HR-pQCT. This cutting-edge technology will be used to demonstrate that T therapy will improve bone strength, and other independent determinants of fractures as microarchitecture and geometry. HR-pQCT is especially clinically relevant because T2D is associated with upper and lower extremity fractures [48] and HR-pQCT is capable of differentiating structural changes at non-weight bearing (radius) vs weight-bearing (tibia) sites. While HR-pQCT and μFEA will provide information on bone structure and simulated strength, micro-indentation will provide valuable information on the bone at the tissue level, i.e., how the mineralized collagen fibril resist fractures in-vivo. Hence, our comprehensive approach will provide a complete picture of how the individual components of bone quality (structure and material) change and interact in response to T therapy. We hypothesize that T therapy improves not only the structural properties but also the intrinsic tissue properties of bone.

This study will provide important information about the changes in bone turnover markers during the T therapy and whether they correlate with changes in circulating osteoblast progenitors and osteoclast precursors and in parameters of bone quality. The main impairment in bone metabolism associated with T2D is that it leads to low bone formation which results in overall low bone turnover [1,49,50]. Bone remodeling is maintained by the normal process of alternating bone resorption and bone formation. An increase in bone turnover is considered undesirable in certain conditions (e.g. sex hormone deficiency) as it is associated with bone loss and deterioration in bone quality; on the other hand, severe suppression of bone turnover also leads to skeletal fragility, e.g. patient with atypical femur fracture from prolonged bisphosphonate use [51,52]. The latter effect is related to the inability to replace an aging bone with new bone and repair microcracks, which normally increases with aging. We previously demonstrated that men with both T2D and hypogonadism have suppressed bone turnover [46]. Meanwhile, Ghanim et al. demonstrated an increase in bone turnover in men with subnormal free T and T2D randomized to T therapy [15]. An increase in bone turnover from T therapy in these patients would mean a positive effect from the treatment, while further suppression of bone turnover would be detrimental. Through the exploratory aim of this study, we will provide important information about the mechanism responsible for the amelioration in bone turnover suppression and improvement in bone quality that we anticipate in response to T therapy. We hypothesize that T therapy will result in stimulation of osteoblastic proliferation and differentiation [16], leading to bone remodeling cycle activation, from the cross-talk between osteoblasts and osteoclasts. This whole process will result in the replacement of old with new bone, and ultimately, in improvement in bone quality. Moreover, it is possible that our T treated subjects will experience an increase in osteocyte number as a result of the increased osteoblastogenesis and of the rise in E2 levels (due to exogenous T converted into E2); the latter an inhibitor of osteocyte apoptosis.

By providing novel data from RCT, results from this study will inform clinical guidelines if T is beneficial or potentially harmful in hypogonadal men with T2D whose bone turnover is relatively suppressed at baseline and will provide information on the utility of T in improving bone quality in these patients in addition to an improvement in quality of life. Given the relationship between glucose metabolism and T production, and the increasing number of male patients diagnosed with both hypogonadism and T2D, this study will benefit not only the significant number of male veterans who have both conditions but also men in general. The conceptual innovation of this study is that T therapy will improve bone quality because of its effect on osteoblast proliferation and differentiation, which in turn leads to activation of osteoclastogenesis, hence bone turnover. The technical innovation lies on the application of novel approaches to comprehensively monitor the mechanical, structural and tissue-level properties of bone (i.e. bone quality beyond BMD) during T therapy in patients with T2D and hypogonadism.

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